Saudi Pharmaceutical Journal xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Saudi Pharmaceutical Journal

journal homepage: www.sciencedirect.com

Original article

In vitro characterization of arylhydrazones of active methylene derivatives

Suresh Palanivel [a], Anastasia Zhurina [a], Phuong Doan [a], Jerome G. Chandraseelan [a], Vinoth Kumar Megraj Khandelwal [c], Fedor I. Zubkov [d], Kamran T. Mahmudov [e,f], Armando J.L. Pombeiro [e], Olli Yli-Harja [b], Meenakshisundaram Kandhavelu [a,*]

[a] *Molecular Signaling Lab, CSB, BioMediTech Institute and Faculty of Biomedical Sciences and Engineering, Tampere University of Technology, P.O. Box 553, 33101 Tampere, Finland*
[b] *Computational Systems Biology Group, BioMediTech Institute and Faculty of Biomedical Sciences and Engineering, Tampere University of Technology, P.O. Box 553, 33101 Tampere, Finland*
[c] *Department of Translational Pharmacology, Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy*
[d] *Organic Chemistry Department, RUDN University, 6 Miklukho-Maklaya St., Moscow 117198, Russian Federation*
[e] *Centro de Química Estrutural, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal*
[f] *Department of Ecology and Soil Sciences, Baku State University, Z. Xalilov Str. 23, Az 1148 Baku, Azerbaijan*

ARTICLE INFO

Article history:
Received 15 October 2017
Accepted 26 December 2017
Available online xxxx

Keywords:
Arylhydrazones of active methylene compounds
Cytotoxic effect
Apoptosis
Immortal cells
Glioma
Single cell analysis
Chemotherapy

ABSTRACT

Arylhydrazones of active methylene compounds (AHAMCs) are potent chemotherapy agents for the cancer treatment. AHAMCs enhance the apoptotic cell death and antiproliferation properties in cancer cells. In this study, a series of AHAMCs, 13 compounds, was assayed for cytotoxicity, apoptosis, externalization of phosphatidylserine, heterogeneity and cellular calcium level changes. The *in vitro* cytotoxicity study against HEK293T cells suggests that AHAMCs have significant cytotoxic effect over the concentrations. Top 5 compounds, 5-(2-(2-hydroxyphenyl) hydrazono)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**5**), 4-hydroxy-5-(2-(2,4,6-trioxo-tetrahydro-pyrimidin-5(6H) ylidene)hydrazinyl)benzene-1,3-disulfonic acid (**6**), 5-chloro-3-(2-(4,4-dimethyl-2,6-dioxocyclohexylidene)hydrazinyl)-2-hydroxybenzenesulfonic acid (**8**), 5-(2-(4,4-dimethyl-2,6-dioxocyclohexylidene)hydrazinyl)-4-hydroxybenzene-1,3-disulfonic acid (**9**) and 2-(2-sulfophenylhydrazo)malononitrile (**10**) were chosen for the pharmacodynamics study. Among these, compound **5** exhibited the better cytotoxic effect with the IC_{50} of 50.86 ± 2.5 mM. DNA cleavage study revealed that **5** induces cell death through apoptosis and shows more effects after 24 and/or 48 h. Independent validation of apoptosis by following the externalization of phosphatidylserine using Annexin-V is also in agreement with the potential activity of **5**. Single cell image analysis of Annexin-V bound cells confirms the presence of mixture of early, mid and late apoptotic cells in the population of the cells treated with **5** and a decreased trend in cell-to-cell variation over the phase was also identified. Additionally, intracellular calcium level measurements identified the Ca^{2+} up-regulation in compound treated cells. A brief inspection of the effect of the compound **5** against multiple human brain astrocytoma cells showed a better cell growth inhibitory effect at micro molar level. These systematic studies provide insights in the development of novel AHAMACs compounds as potential cell growth inhibitors for cancer treatment.

* Corresponding author.
E-mail addresses: suresh.palanivel@student.tut.fi (S. Palanivel), ana.zhrn@gmail.com (A. Zhurina), phuong.doan@tut.fi (P. Doan), cjgeoffrey@gmail.com (J.G. Chandraseelan), drvinothk@gmail.com (V.K.M. Khandelwal), fzubkov@sci.pfu.edu.ru (F.I. Zubkov), kamran_chem@mail.ru (K.T. Mahmudov), pombeiro@tecnico.ulisboa.pt (A.J.L. Pombeiro), olli.yli-harja@tut.fi (O. Yli-Harja), meenakshisundaram.kandhavelu@tut.fi (M. Kandhavelu).

Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

1. Introduction

Arylhydrazones of active methylene compounds (AHAMCs) and their metal complexes have emerged as potential chemotherapeutic drugs for cancer treatment (Hamid et al., 2004; Sambasivarao, 2013; Jimenez et al., 2009; Jones and Senft, 1985; Nitsch et al., 2000; Ozdemir et al., 2003). AHMACs are linking β-diketones and aryldiazonium salt that have been identified as potential antibacterial, antifungal, analgesic and antipyretic drugs (Hamid et al., 2004; Sambasivarao, 2013; Jimenez et al., 2009; Jones and Senft, 1985; Nitsch et al., 2000; Ozdemir et al., 2003; Arndt-Jovin and Jovin,

https://doi.org/10.1016/j.jsps.2017.12.018
1319-0164/

Please cite this article in press as: Palanivel, S., et al. *In vitro* characterization of arylhydrazones of active methylene derivativesIn vitro characterization of arylhydrazones –>. Saudi Pharmaceutical Journal (2018), https://doi.org/10.1016/j.jsps.2017.12.018

1989; Zhang et al., 1997). Recently, Di- and tri-organotin(IV) complexes of arylhydrazones of methylene active compounds are evaluated as potential antiproliferative compounds against in HCT116 and HEPG2 tumor cell lines (Nitsch et al., 2000). Besides, curcumin is a one of the family of β-diketone derivatives extracted from Curcuma longa exhibits tumor suppressive and preventive characteristics in many cancerous models (Demchenko, 2013). Curcumin induces the apoptosis by Bid cleavage, caspase 9 and 3 activation, and by downregulating anti-apoptotic proteins BCl-2 and BclXL (Zhang et al., 1997; Hingorani et al., 2011). The analogues of Curcumin also effectively inhibit the growth of prostate cancer cells. Lanthanide chelation with β-diketones derivatives have also been applied in the treatment of colon cancer (Hagan and Zuchner, 2011). Polyphenol 3 compound is shown to have more toxicity effect on HEK293T cell lines at 48 h than any other compounds that are being used commonly in the cancer treatment (Hamid et al., 2004; Sambasivarao, 2013; Jimenez et al., 2009; Jones and Senft, 1985; Nitsch et al., 2000; Ozdemir et al., 2003; Arndt-Jovin and Jovin, 1989; Zhang et al., 1997; Doan et al., 2016).

However, the mechanism such as transportation of the drug through plasma membrane, defence against drug, enhanced DNA repair, alterations in target molecules, access to target cells, metabolic effects and growth factors, are common barrier that reduce the development of efficient anti cancerous drugs (Helmy and Abdel, 2012). Ultimately, cancer cells protect themselves in a distinctive microenvironment resulting in disrupting the apoptotic mechanism in response to the drugs. This results in the failure of chemotherapy and development of multi-drug resistivity. Thus, the main objective of the chemotherapeutic agents is to progress therapeutic efficacy, selectivity and incapacitating drug resistance for enhanced cancer treatment (Tian et al., 2009).

The chemotherapeutic drugs are having potential's to induce apoptosis in many cancer cells. Severe damage to the genomic DNA by drugs rather leads to the activation of specific regulatory gene that causes apoptosis in a cell (Mattana et al., 1997). Initiating the apoptosis regulatory genes cause the cell to undergo cell morphological changes resulting in programmed cell death. Apoptosis is an active regulatory mechanism, energy-dependent process, plays a vital role in the cancer cell death (Grynkiewicz et al., 1985). It is clearly observable that inducing the apoptotic responsible gene is one of the efficient ways for the cancer cure. The cytotoxicity and apoptosis associated damage are the two significant actions result in chemotherapy induced apoptosis. Thus, the enhanced treatment in chemotherapy helps to develop drugs and to target the essential regulators for the apoptotic activation (Hahnel et al., 1999; Oruç et al., 2006).

Here, we investigate the possibility of cytotoxic effects of novel AHMACs against human embryonic kidney cell line (HEK293T) and multiple glial tumor cells lines. HEK293T cells are suitable for investigating the potential toxicity of nanoparticles too. HEK293 are immortalized already by known oncogene but not malignant yet (Rauf et al., 2008; Küçükgüzel et al., 1999). HEK293T cells were generated by transformation of normal HEK cells with sheared human adenovirus type 5 DNA that results in decreased senescence. At first, we studied the effect of 13 novel AHMACs compounds and top 5 compounds were selected for the evaluation of cytotoxicity over the concentrations and time. To test the induction of apoptosis the Propidium iodide and Annexin-V staining methods were performed. Further, the effect of top compound on cell-to-cell variation and intracellular calcium also measured using microscopic imaging analysis. Additionally, to observe the potential growth inhibitory effect of the top compound on multiple glial tumor cell lines we used 1321N1 and U87-MG. Therefore, this study extends the possibilities to use novel AHMACs against the growth of many cancer cells.

2. Materials and methods

2.1. Synthesis of novel compound azo derivatives of AHMACs

Thirteen novel AHMACs were synthesized and used in this study. The list of compounds are as follows: (*E*)-3-(2-(1-ethoxy-1,3-dioxobutan-2-ylidene)hydrazinyl)-2-hydroxy-5-nitrobenzene sulfonic acid (**1**), 2-(2-(2-hydroxy-4-nitrophenyl)hydrazono)-2*H*-indene-1,3-dione (**2**), (*Z*)-5-chloro-2-hydroxy-3-(2-(4,4,4-trifluoro-1,3-dioxo-1-(thiophen-2-yl)butan-2-ylidene)hydrazinyl)benzenesulfonic acid (**3**), 5-chloro-2-hydroxy-3-(2-(2,4,6-trioxo-tetrahydropyrimidin-5(6*H*)-ylidene)hydrazinyl)benzenesulfonic acid (**4**), 5-(2-(2-hydroxyphenyl) hydrazono)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**5**), 4-hydroxy-5-(2-(2,4,6-trioxo-tetrahydro-pyrimidin-5(6H) ylidene)hydrazinyl)benzene-1,3-disulfonic acid (**6**), 5-(2-(2-hydroxy-4-nitrophenyl)hydrazono)pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (**7**), and 5-chloro-3-(2-(4,4-dimethyl-2,6-dioxocyclohexylidene)hydrazinyl)-2-hydroxybenzenesulfonic acid (**8**) 5-(2-(4,4-dimethyl-2,6-dioxocyclohexylidene)hydrazinyl)-4-hydroxybenzene-1,3-disulfonic acid (**9**), 2-(2-sulfophenylhydrazo)malononitrile (**10**), 2-(2-carboxyphenylhydrazo)malononitrile (**11**), 2-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl)phenylarsonic acid (**12**) and 5-(2-(2,4-dioxopentan-3-ylidene)hydrazinyl)-2,3-dihydrophthalazine-1,4-dione (**13**) were used. The compounds **1**–**8** were synthesized by reaction between the diazonium salt of substituted anilines and β-diketones following Japp-Klingemann reaction. The compounds **9**–**13** were synthesized by reaction between the respective aromatic diazonium salt and methylene active compounds in a water solution containing sodium acetate or sodium hydroxide (Oruç et al., 2006; Rauf et al., 2008; Küçükgüzel et al., 1999; Kandhavelu et al., 2012; Mahmudov et al., 2014; Shukla et al., 2012; Viswanathan et al., 2014; Cells et al., 2011). The synthesis and characterization of AHAMCs (Scheme 1) were reported earlier by us (Kandhavelu et al., 2012).

2.2. Cell culture

Human Embryonic Kidney cells (HEK293T) (ATCC, crl-3216, Sigma-Aldrich, UK) were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% (v/v) of fetal bovine serum (FBS), penicillin and streptomycin (100 U/ml), sodium pyruvate (1mM), and amphotericin B (250 μg/ml) (Sigma-Aldrich, St. Louis, MO) and grown at 37 °C in a humidified 95% air and 5% CO_2. To characterize cytotoxicity of the novel compounds, 1×10^6 cells per well were seeded in a 6-well plate and incubated at 37 °C in the CO_2 incubator (passage 20). After incubation for 24 h, the medium was removed and replaced with fresh medium.

Human brain astrocytoma cell lines U-87 MG and 1321N1 were used for screening the activity of top compounds. Minimum Essential Medium Eagle (MEM) with L-glutamine was used for culturing U-87 MG cells (passage 19). For 1321N1 cells (passage 23), to Dulbecco's Modified Eagle Medium (DMEM) with L-glutamine, 2 mM sodium pyruvate was added. In addition, 10% (v/v) Fetal Bovine Serum (FBS), penicillin and streptomycin (100 U/ml), and 0.025 mg/mL amphotericin B, were added in Both U-87 MG and 1321N1 media. The cells maintained in the incubator with humidified conditions at 37 °C and 5% CO_2.

2.3. Cytotoxicity of AHAMCs

The compounds were dissolved in DMSO (>99.7%) to obtain a concentration of 100 mM. Initial dilution of the compounds was made with DMSO and further dilutions were made using dH_2O. To determine cytotoxicity, first, cells were treated with a maximum concentration of 25 mM of each compound and incubated

Please cite this article in press as: Palanivel, S., et al. *In vitro* characterization of arylhydrazones of active methylene derivativesIn vitro characterization of arylhydrazones –>. Saudi Pharmaceutical Journal (2018), https://doi.org/10.1016/j.jsps.2017.12.018